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Toxicity of Methylenebisthiocyanate (MBT) to Several Freshwater Organisms

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The organo-sulfur compound methylenebisthiocyanate (MBT) has successfully been used as preservative in latex emulsions, starches and gums, acrylic fibers, coatings, curing salts for hide and leather and cutting oil systems (Wehner and Hinz 1971). The greatest application of MBT is for cooling water systems and paper mill systems as effective inhibitor of algae, fungi and bacteria, in particular the sulfate reducing anaerobic Desulfovibrio sp. (Cappeline 1977). Low concentrations of 3-4 mg/L have proved to be successful. For its use as microbiocide in water systems, MBT is usually formulated with dispersants (on basis of alkylglycols) to make it penetrable to invade the slime layers of algae and bacteria (Cappeline 1977; McCoy 1974). Because MBT is not substantive to cellulose or other particulate matter, or to debris in systems, it remains in the water (Wehner and Hinz 1971). However, MBT hydrolyzes rapidly above pH 8.0 (Cappeline 1977; McCoy 1974). The half-conversion time at pH 8.0 is about 4.5 h(Rus 1978). No data are available on residues of MBT in the aquatic environment.

Information on the toxicity of MBT, except for its effects on bacteria and algae, is scarce. Therefore research was carried out to evaluate its risk to aquatic life.

MATERIALS AND METHODS

MBT was supplied by Betz, Herenthals, Belgium (chemical purity 95%). Stock solutions of the test compound were made in acetone (Baker Chemicals B.V., Deventer, the Netherlands; chemical purity >99%) and were prepared fresh each time.

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Short-term toxicity tests with rainbow trout (Salmo gairdneri; 14 days), guppies (Poecilia reticulata; 96 h), water fleas (Daphnia magna; 48 h) and unicellular green algae (Chlorella pyrenoidosa; 96 h) were carried out according to the guidelines of the Organization for Economic Co-operation and Development and the Dutch Standardization Organization. Detailed information about the chemical composition of the media and methods used is given in Van Leeuwen and Maas (1985). The test with algae was carried out in triplicate, the other tests were performed in duplicate. The 14-day toxicity test with rainbow trout was carried out with juveniles of 6 months with a length of approximately 6 cm. The media were renewed three times a week. Fish were fed with Trouvit pellets (Trouw & Co. N.V., the Netherlands) prior to renewing. Test solutions in the 96-hour test with guppies were renewed daily. Tests with daphnids were performed at pH 6.0 and pH 8.0. Daphnids were fed on 1 x 10^8 cells/L C. pyrenoidosa at the beginning of the tests.

Toxicity testing with luminescent bacteria (Photobacterium phosphoreum) was carried out in accordance with the procedure described by Beckman (1982). Effects on nitrifying bacteria (Nitrosomonas and Nitrobacter) were studied with the Blok (1981) screening test. After 3 h the minimum inhibitory concentration (MIC) was determined visually.

Long-term experiments were performed with D. magna (21 days) and S. gairdneri (60 days) according to Van Leeuwen et al. (1985; 1986). The embryolarval test with trout was conducted in duplicate, and initiated with freshly, artificially spawned eggs from a fish hatchery. Within 3h after fertilization egg samples (size 100) were introduced to the aquaria. At the test temperature (10°C) the hatching time for eggs of rainbow trout was 28 days. Acute LC50 values and 95% confidence limits were calculated according to Litchfield and Wilcoxon (1949). Population growth curves of C. pyrenoidosa were analysed according to Kooyman et al. (1983). The calculation of the EC50 and 95% confidence limits for P. phosphoreum was carried out in accordance with the procedure described by Beckman (1982). The intrinsic rate of natural increase (r_m), derived from the long-term experiment with daphnids, was calculated by successive approximation from the formula of Lotka (Van Leeuwen et al. 1985). Effects on the r_m and the length of daphnids were tested using Williams' procedure (1971; 1972) and expressed as lowest rejected concentration tested (LRCT). The LC50 and 95% confidence limits of the long-term tests were determined according to Kooyman (1981). The same procedure was applied to the calculation of the EC50 and 95% confidence limits after combining frequences of embryonic mortality and abnormal development observed in juveniles. Differences in mean survival and normal development at the experimental concentrations were tested against the control by means of a X^2-test (Sokal and Rohlf 1981). Differences in mean length and weight between treatments and control were tested using the procedures described by Williams (1971; 1972) and expressed as LRCT.

RESULTS AND DISCUSSION

The results of the toxicity tests are summarized in Table 1. MBT is highly toxic to most of the species tested. D. magna appears to be the most sensitive species. As the half-conversion time of MBT at pH 8.0 is approximately 4.5 hours (Rus 1982) it can be concluded that in most experiments the species were exposed to hydrolyzed MBT. The short-term toxicity tests with D. magna performed at pH 8.0 and pH 6.0, respectively, show that toxicity decreases at higher pHs. However as the reproducibility of short-term tests with stable compounds lies within a factor 2 to 3 (Canton and Adema 1978) this difference for an unstable compound is probably not significant.

Table 1. Summary of results obtained from short-term and long-term toxicity studies with MBT and several freshwater species.

Species	Criterion	Result and 95% C.L. (µg/L)
Nitrosomonas/ Nitrobacter	nitrification (3h-MIC)	3200
P. phosphoreum	bioluminescense(15min-EC50)	54(49-59)
C. pyrenoidosa	population growth(4d-EC50)	42(27-67)
D. magna	survival (48h-LC50)	25(23-27)[a]
D. magna	survival (48h-LC50)	73(67-79)[b]
D. magna	survival (21d-LC50)	31(28-33)[b]
D. magna	population growth(21d-LRCT)	56[b]
D. magna	individual growth(21d-LRCT)	56[b]
P. reticulata	survival (4d-LC50)	390(330-470)
S. gairdneri	survival (14d-LC50)	84(72-99)
S. gairdneri	survival (60d-LC50)	65(58-72)
S. gairdneri	total embryotoxicity(60d-EC50)	59(51-68)
S. gairdneri	growth (60d-LRCT for length)	100
S. gairdneri	growth (60d-LRCT for weight)	100

[a] pH of test medium: 6.0
[b] pH of test medium: 8.0

Population growth of C. pyrenoidosa at different concentrations of MBT is shown in Figure 1. MBT induces acute mortality relative to its concentration resulting in an increase of the time-lag until maximum population growth.

The results of the life-table experiments with D. magna are shown in Table 2. Specific inhibition of both individual and population growth (r_m) was not observed as MBT at a concentration of 56 µg/L causes almost complete mortality. The effects of MBT are acute as it appears that the LC50 hardly decreases after 48 h of exposure (Figure 2).

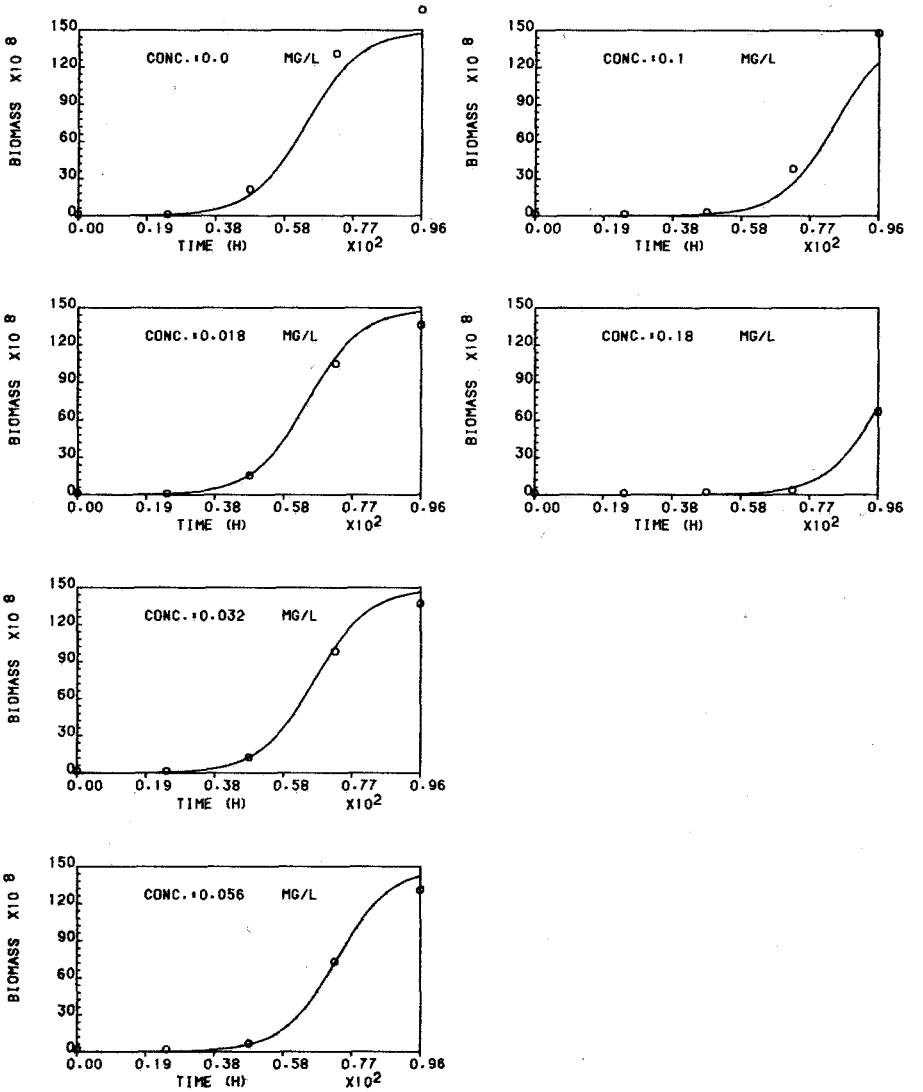

Figure 1. Effects of MBT on the inoculum in a 96-h test with C. pyrenoidosa populations. Circles represent the observed and lines the expected number of algae/L.

The results of the embryolarval test with S. gairdneri are presented in Table 3. In concentrations up to 32 µg/L MBT, teratogenicity was not observed. At 100 µg/L MBT induced congenital defects including scoliotic caudal peduncle, reduced caudal fin and anomalous adipose fin (Figure 3).

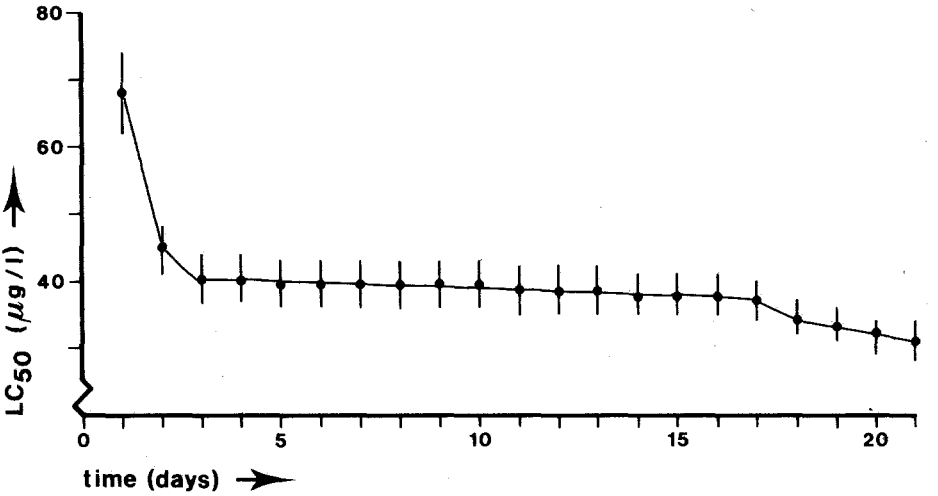

Figure 2. The relation between LC50 and time for D. magna and MBT. Bars represent 95% confidence limits.

Delayed yolk-resorption was also observed at this concentration. The survival pattern of S. gairdneri during embryolarval development (Figure 4) shows that the effects of MBT at 320 µg/L are acute. At 100 µg/L MBT, an increase of mortality was also observed after larval emergence from the eggs.

Little is known about the toxic mechanisms of MBT. Competitive inhibition of the respiratory system in cells, i.e., inactivation of the electron transfer system as a consequence of complexation of the thiocyanate fragment with the ferric ion of cytochrome has been suggested (Cappeline 1977 and McCoy 1974). This cytotoxic mechanism may account for the broad biocidal activity of MBT. Since normal dosages of MBT are in the ppm-range and toxic effects are induced in the ppb-range, MBT may pose a hazard to the functioning of aquatic ecosystems.

Table 2. Results of a reproduction toxicity test with D.magna and MBT.

Concentration (µg/L)	Survival after 21 days (%)	r_m ± S.E.	length ± S.E. (mm)
0	88	0.316 ± 0.008	3.76 ± 0.15
5.6	86	0.319 ± 0.018	3.79 ± 0.15
10	90	0.332 ± 0.011	3.84 ± 0.13
18	82	0.327 ± 0.007	3.79 ± 0.14
32	50	0.321 = 0.006	3.92 ± 0.12
56	2	- [a]	- [a]
100	-	-	-

[a] LRCT ($\alpha<0.01$)

Table 3. Results of an embryolarval toxicity experiment with S. gairdneri and MBT.

Concentration (µg/L)	Mortality (%) egg-stage	larval-juvenile stage	total after 60 days	Mortality and teratogenicity after 60 days (%)	Mean length and 95% C.L. (mm)	Mean weight and 95% C.L. (mg)
0	10.3	3.3	13.6	13.6	27.3 (27.2-27.4)	157.2 (154.1-160.3)
3.2	11.0	3.1	14.1	14.1	27.4 (27.2-27.5)	149.3 (146.6-152.0)
10	11.5	7.1	18.7	18.7	27.4 (27.3-27.5)	151.8 (148.8-154.8)
32	8.8	9.4	18.2	18.2	27.4 (27.3-27.5)	154.0 (150.8-157.2)
100	46.5	41.7	88.2[a]	90.9[a]	19.7 (18.4-21.0)[a]	111.5 (104.7-118.3)[a]

[a]LRCT ($\alpha < 0.01$)

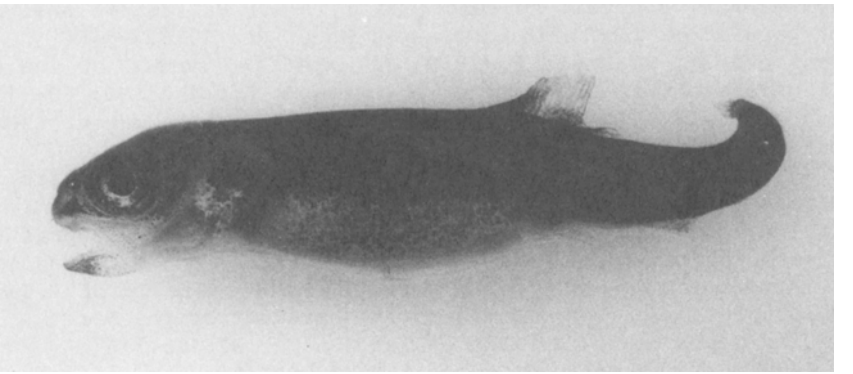

Figure 3. MBT-induced congenital defects including scoliotic caudal peduncle, reduced caudal fin and anomalous adipose fin.

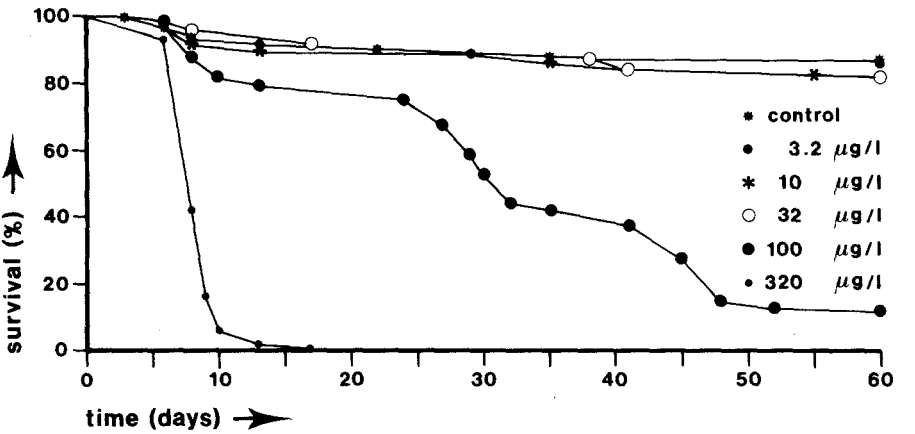

Figure 4. Survival pattern of early life stages of S. gairdneri at various concentrations of MBT.

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